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Research Article

Pharmacognostic, phytochemical, physicochemical and fluorescence analysis of *Terminalia bellerica* leaf and stem

Disha Menpara, Dishant Desai and Sumitra Chanda *

Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University - Rajkot, 360 005, Gujarat, India

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ABSTRACT

To evaluate the pharmacognostic characters of important medicinal plant *Terminalia bellerica* Roxb. Micro and macroscopic characters of fresh and dried leaf and stem were analyzed. Physiochemical analyses were done by using WHO recommended parameters and fluorescent behavior of the leaf and stem powder were also tested. Microscopic studies of leaf revealed the presence of cuticle layer, trichomes, calcium oxalate and starch granules and in stem showed epidermis, narrow cortex, and cambium. Physiochemical parameters such as ash values, loss on drying, extractive values, fluorescence analysis were also determined. Preliminary phytochemical screening showed the presence of flavonoid, tannins and triterpenes. Various pharmacognostical characters were observed in study which can help in identification and standardization of *T. bellerica*.

Key words: *Terminalia bellerica*, pharmacognostic, phytochemical, physicochemical, fluorescence, bahera

INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest sources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties**.** In India, the use of medicinal plants is a century old tradition and approximately two million traditional health practitioners still use medicinal plants for curing various ailments.

According to World Health Organization (WHO) more than 80% of the world's population relies on herbal medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Herbal formulation involves use of fresh or dried plant part. Therefore proper and correct identification of the plant material is very much essential. Correct identification of the starting material is an essential prerequisite to ensure reproducible quality and will contribute immensely to its safety and efficacy (Thomas *et al*., 2008).

Adulteration or substitution is nothing but replacement of original plant material with another plant material or intentionally adding any foreign substance to increase the weight or potency of the product or to decrease its cost. Pharmacognostic studies are pivotal in herbal technology as it ensures plant identity, lays down standardization parameters which will help and prevent adulterations. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products. Similar work has been reported by other researchers in many plants like *Nelumbo nucifera* Gaerth leaf (Jain and Rajput, 2012), *Terminalia catappa* leaf (Kadam et al. 2011), *Polyalthia longifolia* leaf (Dave et al., 2010), *Psidium guajava* L. leaf (Kaneria and Chanda, 2011), etc.
Combretaceae is a large family of plants, the most commonly occurring genera of which are *Combretum* and *Terminalia* (Hutchings *et al.,* 1996), both widely used in traditional medicine. The genus *Terminalia* is widely distributed and is known as a rich source of secondary metabolites (Cao *et al*., 2010; Garcez *et al*., 2003;Mahato *et al.,* 1992; Singh *et al*., 2002) and flavonones and chalcones (Garcez *et al*., 2006).

***Corresponding Author Address:** Dr. Sumitra Chanda, Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University - Rajkot, 360 005, Gujarat, India; E-mail: svchanda@gmail.com*

Terminalia bellerica Roxb. belonging to the family Combretaceae, commonly known as myrobalan, is a deciduous tree found throughout the Indian forests and plains. It is known as Bahera in India and has been used for centuries in Ayurveda, a holistic system of medicine originating from India. The tree is about 30-40 m. in height and 2-3 m. in girth. The stem is straight and the leaves are broadly elliptic clustered near the end of the branches. The flowers are simple, solitary in axillary spikes. The fruit is ovoid 1-2 cm in diameter drupe of grey to dark brown in colour. Fruit extract used as astringent, antiseptic, rejuvenative, brain tonic, expectorant and laxative. It is used in coughs and sore throat. Its pulp used in dysentery, diarrhoea and liver disorders. It is also useful in leprosy, fever and hair care. This plant exhibits several pharmacological effects including antibacterial, antimalarial, antifungal, anti HIV, antioxidant and antimutagenic effects (Bajpai *et al*., 2005). The fruit possesses antidiabetic and antioxidant activities (Sabu *et al*., 2009).

In the present study, an attempt has been made to lay down some standardization parameters for *T. bellerica* leaf and stem. Hence, the objectives of the study were to evaluate various pharmacognostic parameters like macroscopic and microscopic characters, phytochemical and physicochemical characterization including fluorescence study.

MATERIALS AND METHODS

T. bellerica leaf and stem was collected from Rajkot, Gujarat, India in July, 2012. The plant was compared with voucher specimen (voucher specimen number PSN290) deposited by Dr. PS Nagar at the Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The plant parts were washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottles.

Macroscopic study: For morphological observations, fresh leaf and stem was collected from Rajkot, Gujarat, India in August, 2012. The macromorphological feature of the leaf and stem was observed under magnifying lens (Tyler *et al.*, 1977).

Microscopic study: Free hand section of leaf and stem was taken and stained by safranine to confirm its lignifications. Powder microscopy was also carried out and the specific diagnostic characteristics were recorded (Khandelwal, 2008).

Phytochemical analysis:

Qualitative phytochemical analysis: The crude powder of leaf and stem was subjected to qualitative phytochemical analysis (Harborne, 1973). The phytochemicals analysed were alkaloids, flavonoids, tannins, phlobatanins, triterpenes, steroids, saponinis and cardiac glycosides.

Fluorescence analysis: Fluorescence study of leaf and stem powder was performed as per reported standard procedures (Kokashi *et al.*, 1958). A small quantity of the stem powder was placed on grease free clean microscopic slid and 1-2 drops of freshly prepared reagent solution was added, mixed by gentle tilting of the slide and waited for few minutes. Then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365 nm) ultra violet radiations. The colour observed by application of different reagents in different radiations was recorded.

Physicochemical analysis: The physicochemical analysis of the crude powder *T. bellerica* leaf and stem was carried out as per WHO guidelines (WHO, 2002). The parameters analysed were Loss on drying, Total Ash ,Water soluble ash, Acid insoluble ash, Petroleum ether soluble extractive, Ethyl acetate soluble extractive, Acetone soluble extractive, Water soluble extractive.

RESULTS AND DISCUSSIONS

Pharmacognostic study

Organoleptic and macroscopic characteristics: The organoleptic and macroscopic features are shown in Table 1. The fresh leaves were light or pale green in colour. The leaves are 10-20 cm in length and 7-15 cm in width. They are acuminate at the apex, base narrowed and cuneate, emarginated, both surfaces puberulous when young, glabrous and reticulate when old, margins entire, subcrenulate pellucid. The venation is reticulate. Macroscopic evaluation is a technique of qualitative evaluation based on the study of morphological profile of the drug. These characters can serve as diagnostic parameters.

Microscopic characteristics: The transverse section of *T. bellerica* leaf showed presence of upper and lower epidermis. The epidermis was covered with a single layer of cuticle; unicellular trichomes were present. The vascular bundle was surrounded by 3-5 layers of cortex. Xylem was lignified while phloem was non lignified. The pith was made up of large cells. Cluster and rosette crystals of calcium oxalate and starch granules were also present (Fig. 1). The transverse section of *T. bellerica* stem showed that stem is angled; on maturation each goes deep inside forming sharp pointed like projection and shows single layer of epidermis, narrow cortex, cambium and

endodermis near above surrounded by medullary rays inside vascular tissue, proto xylem and meta xylem are centrally located on phloem (Fig. 2).

Powder study: The crude powder of *T. bellerica* leaf was green in colour. The dignostic features of powder were cluster and rosette type of crystals of calcium oxalate. Trichome and xylem were also observed. (Fig.3). The fine powder of *T. bellerica* stem was brown in color with faint odour. The diagnostic features of powder were scalariform xylem vessel, unicellular trichome, cluster crystal and rosette crystal (Fig. 4). The main diagnostic microscopic features were angled stem and presence of cluster and rosette crystals.

Phytochemical analysis: The plants are considered as biosynthetic laboratory for a multitude of compounds that exert physiological effects. Secondary metabolites are the compounds which are responsible for imparting therapeutic effects. The preliminary phytochemical analysis will give an idea about the chemical nature of the drug**.** The information obtained will be useful in further structural characterization of the nature of constituents present in the plant material under investigation. It could be also helpful to extract out particular constituents by a particular solvent. The preliminary qualitative phytochemical investigation of *T. bellerica* leaf and stem was performed which shows the presence of flavonoids, tannins and triterpenes. They were devoid of alkaloids. steroids, saponins and cardiac glycosides (Table 2).

Fluorescence analysis: The fluorescence character of powdered drug plays a vital role in the determination of quality and purity of the drug material. In the present study, leaf and stem powder treated with various reagents showed characteristic fluorescence at 254 nm and 366 nm wavelength (Tables 3 and 4). Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence crude drugs are often assessed qualitatively in this way and it is an important parameter for pharmacognostic evaluation of crude drugs (Kumar and Kumar, 2012; Zhao *et al*., 2011).

Physicochemical study: Various physico-chemical parameters of powdered drug are shown in (Table 5). Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage.

The extractive values (Table 5) are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. Higher extractive value of petroleum ether extract is due to the presence of fixed oil and fat. Water soluble extractive value is higher than any other solvent, which indicates the presence of highly polar chemical constituents such as flavanoids, proteins, carbohydrates, etc.

Such data is useful in identification and standardization of plant drug and in this study it is *T. bellerica* leaf and stem and such standardization parameters will help in identifying the drug even in the powdered form and it can be easily distinguished and identified from adulterants. Thus such parameters are helpful and are of great value in the quality control and formulation development. Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. The microscopic characters, the physiochemical studies and Fluorescence analysis can be used for the quality control of the crude drug. Such a pharmacognostic study is useful for standardizing crude drugs and can be used to differentiate closely related species. This could also serve in establishing the data for preparation of monograph of this plant. Various physicochemical parameters established can be important in detecting adulteration and mishandling of the crude drug.

In conclusion, the parameters which are described here can be considered as distinctive characters of this plant and are good enough to authenticate the drug in herbal industry and prevent adulteration. This will also aid in maintaining the quality assurance of the starting material.

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Table 1: Organoleptic features of *T. bellerica* leaf

Colour	Light/pale green
Odour	Characteristic
Taste	Astringent
Shape	Broadly elliptic and/or elliptic-o
Dimensions	Length-10-20 cm, Width-7-15 cm
Leaf Surface	Puberulous when young, glabrous and reticulate when old
Margin	Entire
Apex	Acuminate
Base	Narrowed and cuneate
Venation	Reticulate
Inflorescence	5-10 cm long, axillary solitary or clustered spikes

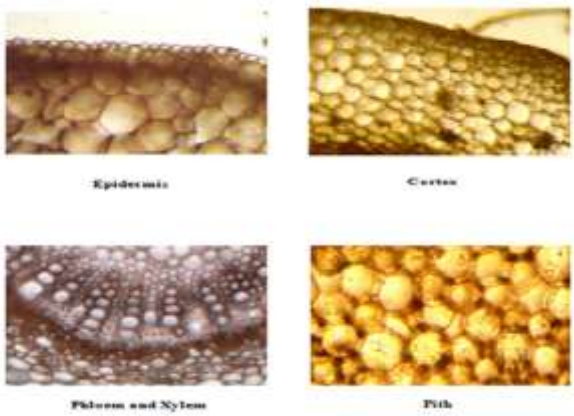

Fig.1 Photomicrographs of microscopic characteristics of *T. bellirica* leaf

Fig.2 Photomicrographs of microscopic characteristics of *T. bellerica* stem

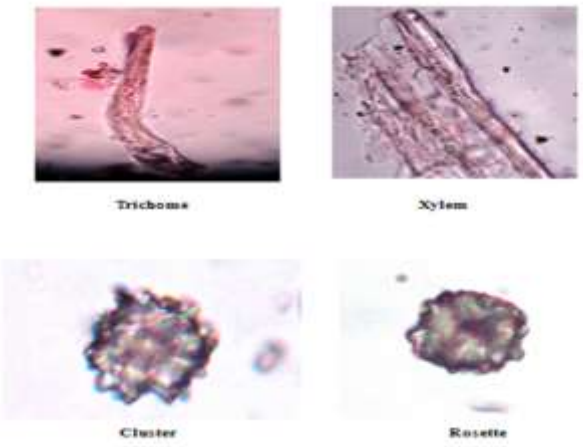

Fig.3 Photomicrographs of microscopic characteristics of powder of *T. bellerica* leaf

Fig.4 Photomicrographs of microscopic characteristics of powder of *T. bellerica* stem

Table 2: Qualitative phytochemical analysis of *T. bellerica* (gaerth.) Roxb leaf and stem.

Phytochemicals		**Leaf**	**stem**
Alkaloids	Hager	-	-
	Mayer	-	-
	Wagner	-	-
Flavonoids		+	+
Tannins		+	+
Cardiac glycosides		-	-
Triterpenes		+	+
Steroids		-	_
Saponins		-	_

Table 3 Fluorescence analysis of *T. bellerica* leaf powder

Treatment	Visible light	Under UV light Short Wavelength (254 nm)	Long wavelength (365) nm
Powder + 1 N NaOH (aq)	Light brown	Black	Light green
Powder + 1 N NaOH (alc)	Greenish brown	Black	Green
Powder + Ammonia	Greenish yellow	Black	Green
Powder +Picric acid	Greenish yellow	Dark coffee	Black
Powder +Petroleum ether	Green	Dark brown	Green
Powder + 50% HCl	Light green	Black	Light green
Powder + 50% H_2SO_4	Dark green	Black	Dark green
Powder + Ethyl acetate	Green	Black	Light green
Powder + Ethylalcohol	Light green	Black	Dark green
Powder + Methanol	Green	Green	Green

Table 4: Fluorescence analysis of *T. bellerica* stem powder

Treatment	Visible light	Under UV light Short Wavelength (254 nm)	Long wavelength (365 nm)
Powder + 1 N NaOH (aq)	Darkbrown	Black	Dark green
Powder + 1 N NaOH (alc)	Brown	Black	Green
Powder + Ammonia	Reddish brown	Black	Green
Powder + Picric acid	Brownish yellow	Dark coffee	Black
Powder + Petroleum ether	Brown	Light brown	Green
Powder + 50% HCl	Light brown	Black	Greenish brown
Powder + 50% H_2SO_4	Light green	Black	Light green
Powder + Ethyl acetate	Light brown	Black	Light green
Powder + Ethyl alcohol	Light brown	Black	Light green
Powder + Methanol	Brown	Green	Green

Table 5: Physiochemical parameters of *T. bellerica* leaf and stem

Parameters	**% Value (w/w) leaf**	**% Value (w/w) stem**
Loss on drying	11.0	8.5
Total ash	18.0	5.25
Water soluble ash	1.83	0.46
Acid insoluble ash	11.7	0.05
Petroleum ether soluble extractive value	0.9	3.0
Ethyl acetate soluble extractive value	2.5	0.7
Acetone soluble extractive value	6.1	1.9
Aqueous soluble extractive value	20.5	5.7

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